Genetic Consequences of Human Forest Exploitation in Two Colobus Monkeys in Guinea Bissau

Tania Minhós[1,2,3*], Lounès Chikhi[3,4], Cláudia Sousa[1,2,] Luis M. Vicente[5], Maria Ferreira da Silva[6,7], Rasmus Heller[3,8], Catarina Casanova[9] and Michael W. Bruford[6]

[1.] Department of Anthropology, Faculdade de Ciências Sociais e Humanas, Universidade Nova de Lisboa, Avenida de Berna, 26-C / 1069-061 Lisbon, Portugal
[2.] Centre for Research in Anthropology, Av. Forças Armadas Ed. ISCTE - sala 2n7, cacifo 237 1649-026 Lisboa Portugal[3]. Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 2780-156 Oeiras, Portugal
[4.] CNRS, EDB (Laboratoire Evolution et Diversité Biologique), UMR CNRS/UPS 5174, Toulouse, France
[5.]Centre for Environmental and Marine Studies, Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, C2-P3 Campo Grande, Lisboa, Portugal
[6.]Organisms and Environment Division, Cardiff School of Biosciences, Museum Avenue, CF10 3AX, Cardiff, UK
[7.] Research Center in Biodiversity and Genetic Resources, Universidade do Porto, Campus Agrário de Vairão, 4485-661, Vairão, Portugal
[8] Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark.
[9] CAPP: Centro de Administracao e Politicas Publicas and Instituto Superior de Ciencias Sociais e Políticas da Universidade de Lisboa, Rua Almerindo Lessa, Polo Universitario do Alto da Ajuda, 1349-055 Lisbon, Portugal

*Corresponding author
Tânia Minhós
Address: Population and Conservation Genetics, Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 2780-156 Oeiras, Portugal
Phone: +351 91 675 42 88
Email: trodrigues@igc.gulbenkian.pt (TM)

Running head: Genetic patterns in sympatric colobus monkeys

Abstract

The ability of forest-dwelling species to adapt to changes in their habitat is being increasingly challenged by the rapid pace of human-induced forest degradation. Understanding the effect of such environmental changes on biodiversity requires comparative analyses across species living within the same habitats. We investigated the effect of forest exploitation on the genetic structure and demography of two sympatric arboreal primates showing differences in their socioecology: the Western black-and-white colobus (*Colobus polykomos*) and Temminck's red colobus (*Procolobus badius temminckii*). We conducted the study in a fragmented and human-impacted forest in Guinea Bissau. Using microsatellite data from six *C. polykomos* and eight *P. b. temminckii* social groups, we found that in *C. polykomos* the distribution of genetic diversity followed an isolation-by-distance pattern whereas for *P. b. temminckii*, the results

suggested restriction in female dispersal. We detected a strong, recent bottleneck for both primates, which we inferred to have resulted from the anthropogenic exploitation of forest resources in the last centuries. The bottleneck signal was stronger for *P. b. temminckii* as a likely consequence of its larger estimated ancestral population size. Finally, we discussed the different analytical approaches used. Our results confirm that *P. b. temminckii* is more affected by habitat changes than *C. polykomos*, despite being phylogenetically close. Nonetheless, the low estimated effective population sizes and the known demographic changes indicate that both species are severely threatened by human forest exploitation, requiring urgent conservation action.

Key-words: primates, non-invasive genetics, demographic bottleneck, genetic structure, fine-scale spatial analysis

1. Introduction

Habitat loss and fragmentation are among the main current threats to biodiversity. Although natural processes can cause population fragmentation and affect population structure, expansion of human land use is occurring at such a pace that most natural populations are likely to be negatively affected (Craul *et al.* 2009; Epps *et al.* 2005; Fahrig 2003; Gerlach & Musolf 2000; Goossens *et al*. 2006). Besides reducing the total area of suitable habitat, the negative effects of landscape modification include habitat fragmentation, a process that divides populations into smaller, isolated units (Frankham *et al*. 2002; Frankham 2006). As a result, a decrease of gene flow and genetic diversity is expected, reducing the evolutionary potential of the population or species and increasing its risk of extinction (e.g. Frankham *et al*. 2002; Johansson *et al*. 2007; Keller & Waller 2002). Genetic data can provide insights into the past and current challenges faced by fragmented wild populations (Chikhi & Bruford 2005; Dixon *et al.* 2007; Goossens *et al*. 2005; Mona *et al.* 2014; Quéméré *et al*. 2010; Zhu *et al*. 2010), and studying the spatial distribution of genetic variation potentially allows the identification of habitat discontinuities responsible for the disruptions of demographic structure (Chikhi & Bruford 2005; Manel *et al*. 2003). Inferring demographic history also allows us to identify major factors (e.g. climatic, ecological, anthropogenic) that have impacted the population in the past and that are likely to influence its distribution in the future (Goossens *et al*. 2005; Olivieri *et al.* 2008; Quéméré *et al.* 2012; Sharma *et al.* 2012).

The extent to which habitat loss and fragmentation affect a species is highly dependent on its ability to disperse, the degree of habitat fragmentation and the matrix of unsuitable habitat between patches (Frankham *et al*. 2002; Frankham 2006). Species that are capable of maintaining dispersal despite habitat fragmentation will be the least affected (Debinski & Holt 2000; Kareiva 1987; Villard 2002). On the other hand, less mobile species or those highly dependent on habitat fragments are more likely to experience isolation (e.g. Goossens *et al*. 2006; Liu *et al*. 2009). Indeed, where biodiversity is concerned, fragmentation is not a simple property of the habitat but rather the result of complex interactions between changes in the habitat and the ability of species to cope with these changes. Comparative analyses across species living in the same habitats are thus necessary. African colobines are expected to be susceptible to habitat fragmentation since they are forest-dependent species. Moreover, and especially

in West African forests, they are declining due to the loss of tropical rainforest, disease outbreaks and ongoing hunting (Minhós *et al.* 2013a; Oates *et al.* 2008a; Oates *et al.* 2008b; Struhsaker 2005). It is true that major population declines, due to habitat loss and human activities, are affecting many West African species, which are under more intense threats when compared to species in other sub-Saharan countries (Brashares *et al.* 2001). For instance, the decline of large mammal populations is higher in West African protected areas than in protected areas from other African regions (Craigie *et al.* 2010). The great conservation challenges faced by West African species are most likely a consequence of various causes acting at various scales, including the lack of financial and personnel resources in protected areas combined with intense hunting for bushmeat consumption (Brashares *et al.* 2004) and habitat destruction. For example, the West African red colobus, *Procolobus badius waldroni,* was the first primate to be declared extinct in the 20th century (Oates *et al.* 2000). These authors claimed that colobus monkeys have been neglected in terms of conservation actions and stressed the urgency for conservation policies that protect the red colobus, otherwise extinction may extend to other subspecies of this primate as well.

The western black-and-white colobus (*Colobus polykomos*) and Temmink's red colobus (*Procolobus badius temminckii*) are the westernmost taxa within the African colobus monkeys. They share many ecological requirements having predominantly arboreal lifestyles within tropical woodlands and are often found in sympatry (Oates *et al.* 1994). However, they have contrasting social systems and differ in their socioecology, which make them interesting models to test the ability of arboreal species to adapt to habitat degradation. *C. polykomos* live in relatively small groups with either males or both sexes dispersing (Korstjens *et al.* 2002; Minhós *et al.* 2013b; Oates *et al.* 1994) and *P. b. temminckii* live in much larger groups with female-biased dispersal (Starin 1994; Minhós *et al.* 2013b). A survey of the eastern black-and-white and red colobus (*Colobus guereza* and *Procolobus pennantii* ssp., respectively) in a highly fragmented habitat in Uganda revealed that red colobus were more sensitive to habitat fragmentation, as they were absent from most forest patches (Onderdonk & Chapman 2000). The ability of black-and-white colobus to adapt their diet, home range and group size to shrinking forest patches was highlighted as a possible explanation for their higher resilience to such degraded forests. Similar flexibility has also been described for the same species in Ethiopia (Dunbar 1987) and Kibale (Uganda; Struhsaker 1997). In Guinea Bissau, *C. polykomos* and *P. b. temminckii* group home ranges often overlap (Gippoliti & Dell'Omo 1996; Gippoliti & Dell'Omo 2003). Both species are reported to have patchy but broad distributions in the country (Gippoliti & Dell'Omo 2003), but the most recent census indicates that they are disappearing from most areas (Casanova & Sousa 2007). Cantanhez National Park (CNP) in South-West Guinea Bissau is one of the last areas in the country where tropical forest—although highly fragmented—still persists and is home to the largest populations of these primates (Fig. 1; IBAP 2007). Nevertheless, forests are being reduced at a rapid rate as a result of human activities and both primates are hunted for meat consumption and commerce (Costa *et al.* 1013; Hockings & Sousa 2013; Minhós *et al.* 2013a).

Here, we tested the hypothesis that forest exploitation (e.g. forest fragmentation, habitat degradation, poaching) has a major negative impact on the persistence of these forest-dwelling primates. As arboreal species, we expected both primates to be affected by forest fragmentation and habitat degradation in Cantanhez. However, we predicted

that *P. b temminckii* is more heavily affected due to female dispersal at shorter distances and its lager social groups when compared with *C. polykomos*. Hence, we investigated the demographic history and the interaction between genetic structure and habitat degradation in CNP for these two sympatric species, using non-invasive molecular techniques. First, we analysed population genetic structure and relatedness patterns at a fine spatial scale to infer discontinuities in dispersal. Second, we inferred and dated changes in effective population size. Finaly, we provide a brief but comprehensive discussion on the advantages and limitations of three different and complimentary methods that we have applied to investigate the demographic history of these species. This comparative study provides new data regarding the interaction between socioecology and a species' capacity to stably persist under anthropogenic habitat disturbance, while also informing upon the conservation priorities that are critical for these threatened populations.

2. Material and Methods

2.1 Study Site and Social Groups

Cantanhez National Park is located on a peninsula (total area: 1 067 Km^2), in south-western Guinea-Bissau (NE limit: 11°22'58''N, 14°46'12''E; SW limit: 11°2'18''S. 15°15'58''W). The forest is now fragmented into patches (ranging from 47.5 to 2,500 ha; Simão 1997) most of which are connected in some way (Fig. 1). Before being declared a National Park in 2008, Cantanhez forest areas were considered critical for biodiversity conservation in Guinea-Bissau, with some of the fragments being protected from extensive exploitation (hunting and logging) by local people. Despite being a National Park where logging activities and hunting are illegal, the national management authority (IBAP – Institute for the Biodiversity and Protected Areas) has limited activity in the park due to financial constraints. As a result, deforestation and hunting remain threats to most wildlife in the park, including the primate species (Ferreira da Silva 2012; Hockings & Sousa 2013; Minhós 2012).

We conducted surveys in 11 forest patches in CNP, between February 2009 and April 2010. Patches where we could not find both species in the first visit were revisited up to 5 times until absence from the area could reasonably be presumed. We targeted the collection of up to ten and 30 fecal samples of each *C. polykomos* and *P. b. temminckii* social group, respectively. We set these limits to assure that the sampling effort was financially viable but that a significant part of each social group was represented and we could sample several social groups of each species. After collecting faecal samples, they were stored using the two-step approach (Roeder et al. 2004). We included in the final dataset the social groups that could represent most of the species distribution in the park (Fig 1).

All sampling was carried out inside the boundaries of Cantanhez National Park, with the approval and permits under the legal requirements of the National Institute for Biodiversity and Protected Areas (IBAP) and the Guinea Bissau Forestry and Fauna Department (DGFC). This was an observational study in that there was no physical interaction with the primates.

2.2 DNA Extraction and Microsatellite Genotyping

Detailed description of the DNA extraction procedure, PCR amplification of 15 microsatellite loci, molecular sex-identification and methods used to deal with genotyping errors are described in Minhós and colleagues (2013b). Screening for null alleles was conducted in Micro-Checker (van Oosterhout 2004) and exact tests for Hardy-Weinberg and genotypic linkage disequilibrium were performed using FSTAT v2.9.3.2 (Goudet 2001). When sampling social groups, the presence of highly related individuals may influence population substructure resulting in a deficit of heterozygotes (Chikhi & Bruford 2005). We assessed pairwise relatedness through a maximum-likelihood (Queller and Goodnight 1989) relatedness estimator using Kingroup v2_101202 (Konovalov *et al*. 2004), where only the significantly related dyads ($p < 0.05$) were considered to be truly related.

2.3 Genetic Structure at the Population Level

We investigated population genetic structure using two Bayesian model-based approaches and compared the consistency of results. These approaches use multi-locus genotypes to cluster individuals into entities that minimize Hardy-Weinberg and linkage disequilibrium, so any departure from random mating may lead to subdivision of the data into sub-populations (Beaumont & Rannala 2004; Pritchard *et al*. 2000). We first used STRUCTURE 2.2 (Pritchard *et al*. 2000), without a spatial prior, to infer the optimal number of genetic clusters (K). We allowed K to range from one to ten, using five independent runs for each K, with 1 000 000 Markov chain Monte Carlo (MCMC) iterations after a 100 000 step burn-in period. Runs were performed under the admixture model and assuming correlated allele frequencies. We used the Structure Harvester tool to access the most appropriate K – number of genetic clusters - chosen following the summary statistic ΔK described by Evanno et al. (2005). Secondly, we used a spatially explicit approach using the software BAPS 5 (Corander *et al*. 2004). We ran BAPS using individual spatial clustering with five replicates varying the maximum K value at 5, 10, 15 and 20. The value of K that best explains the genetic partition of the data is given by its highest probability. When sampling social groups, the presence of highly related dyads of individuals in the sample may induce family-based structure that does not correspond to a true population (Anderson & Dunham 2008; Bergl & Vigilant 2007; Pritchard & Wen 2004). This was the case of the *P. b. temminckii* population, for which the inclusion of related individuals in the dataset produced a strong family-based structure (Fig A1). In order to control for this bias, we ran both methods with a restricted dataset where one individual belonging to each significantly related dyad was removed (final dataset for *C. polykomos*: 22 individuals and *P. b. temminckii*: 23 individuals; hereafter referred to as 'non-related' dataset).

2.4 Fine-scale Genetic Structure

We investigated fine-scale spatial genetic structure by analysing isolation-by-distance and spatial autocorrelation using the entire genetic dataset and for the female and male subset of dyads separately. We used GenAlEx 6.41 (Peakall & Smouse 2006) to

perform a Mantel test to estimate the correlation between the Queller and Goodnight relatedness estimator (Queller & Goodnight 1989) and geographical distance. The significance was assessed by 9 999 permutations. We compared the spatial autocorrelation of individual genotypes with those from areas at set distance intervals. Any deviation of these relationships from zero indicates that individuals are more (positive values) or less (negative values) related than expected at random. We conducted this analysis using SPAGeDI 1.3 (Hardy & Vekemans 2002) and chose spatial categories based on the equal distribution of the number of dyads within ten categories for *C. polykomos* and eight for *P. b. temminckii*. Permutation tests (10 000) were conducted to establish 95% confidence intervals. The first distance class corresponded to a comparison of dyads within the same social group. All other classes comprised individuals belonging to the most adjacent group.

2.5 Demographic History

Demographic changes leave traces in the genome that can be detected through the distributions of allele size and in the shape of the genealogy of microsatellite loci (Beaumont 1999; Cornuet & Luikart 1996; Garza & Williamson 2001; Goossens *et al*. 2006). We used three different and complementary methods (see Discussion) to assess whether the colobus populations from CNP have undergone demographic changes.

Demographic Test #1

We tested for heterozygosity excess by applying the Wilcoxon's signed rank test (10^5 replications), as implemented in BOTTLENECK v1.2.02 (Cornuet & Luikart 1996; Piry *et al.* 1999). We tested the infinite allele (IAM), stepwise mutation (SMM) and two-phase (TPM) models, assuming 95% and 78% of single step mutations with a variance set to 12, following recommendations by Piry *et al.* (1999) and Peery *et al.* (2012), respectively.

Demographic Test #2

We estimated the posterior distribution of demographic parameters using Markov Chain Monte Carlo simulations in the software MSVAR 1.3 (Storz & Beaumont 2002). This hierarchical Bayesian method assumes a simple demographic model entailing a single point of change in population size and estimates the current and ancestral effective population sizes, N_0 and N_1, respectively, as well as the time T (in generations) since the size of the population started to change, assuming an exponential size change. The model assumes lognormal prior distributions for the parameters N_0, N_1, T and μ and that the genetic markers are microsatellites evolving under a stepwise mutation model (SMM). Assuming a generation time of 5 years for both species (Allen *et al.* 2012), T was converted into years for all the analyses. We conducted four independent runs for each data set, varying the prior and hyperprior distributions in order to place most of the prior support on different scenarios of constant population size (run 1: $N_0 = N_1$), bottleneck (run 2: $N_0 < N_1$) and demographic expansion (runs 3 & 4: $N_0 > N_1$)(Table A1). Each run was performed for 9×10^9 iterations with parameter values sampled every 3×10^5 iterations, resulting in 30 000 samples from the posterior

distribution. We discarded the first 10% of each independent simulation (burn-in) to avoid bias in the parameters estimation due to starting conditions. We tested for convergence between simulations both visually and using Brooks, Gelman, and Rubin Convergence Diagnostic test (Brooks & Gelman 1998; Gelman & Rubin 1992;) conducted in R version 2.11.1 (R Development Core Team 2010) using the package BOA version 1.1.7 (Smith 2007). Results from the four independent runs were analysed individually and with all simulations pooled together in one dataset (overall) in order to produce larger and more precise samples of posterior distributions. We also calculated the mean ratio (r) N_1/N_0 over all recorded sample steps as a way to interpret the strength of evidence for various demographic histories (r = 1 indicates constant population size, r > 1 indicates population expansion and r< 1 indicates demographic bottleneck). We used a Bayes Factor analysis (BF, Olivieri *et al.* 2009; Girod *et al.* 2011; Quéméré *et al.* 2012) to test in which time frame the population size change was more likely to have occurred. This approach estimates the ratio of the posterior distribution of two alternative hypotheses over the prior distributions of the same hypotheses, i.e. the ratio of likelihoods over a given parameter space. A BF of 1 indicates that H1 and H2 are equally probable. We defined five time intervals based on different hypotheses regarding the most important factor driving colobus demographic history. These hypotheses are linked to human impact or different climatic epochs that may have led to forest retraction or expansion. The five time intervals were defined as follows: H1 (0 - 200 yrs) – recent anthropogenic forest exploitation; H2 (200 – 1 000) – human colonization of CNP; H3 (1 000 – 6 000) – sequence of dry periods in West Africa; H4 (19 000 – 27 000) – last glacial maximum; H5 (27 000 – 100 000) – ancient events. Note that we did not include the period between 6 000 and 19 000 years in the hypotheses we tested because we found no detailed evidence in the literature of relevant events that might have impacted colobus populations in West Africa. Nevertheless, this period is automatically considered when running the analyses in which we divided time in 10- and 100-years periods and looked at how the BFs changed over time (Olivieri *et al.* 2009).

Demographic Test #3

We inferred the demographic history of the two species using a semi-parametric demographic model and the microsatellite data using the Extended Bayesian Skyline Plot method (EBSP, Heled & Drummond 2008) as implemented in BEAST v1.7.4 (Drummond & Rambaut 2007). For both species we used the 'non-related' reduced data set to avoid a non-random population sample and to decrease computation time. Due to very slow convergence under more complex mutation models in trial runs, we decided to use a simple stepwise mutation model. This also facilitates comparison with demographic test #2. The mutation rate was fixed at 5×10^{-4} per generation, representing the midpoint of the commonly assumed interval of 10^-4 to 10^-3 (e.g Storz et al. 2002). We chose to fix the rate to reduce the MCMC dimensionality, because preliminary analyses suggested that the BEAST runs showed poor mixing and convergence. Note that the dating of demographic changes scales linearly with the mutation rate, so uncertainty in the rate can be reflected simply as a proportional move of the entire EBSP, but would not change the shape of the inferred EBSP. The prior for the demographic.populationSizeChanges parameter was left at the default Poisson distribution with mean ln(2), which is a relatively conservative prior giving 50% weight

to zero demographic changes. The prior for the historical mean of the population size parameter (actually Ne x τ) was uniform between 10^4 and 2 x 10^6. All other priors were left at default values. The MCMC chain was run for 10^8 steps sampling every 10^4 steps, and 3 x 10^7 steps were discarded as burn-in upon visual inspection of the MCMC trace using TRACER v1.5.0 (Rambaut & Drummond 2003). This ensured that most parameter posteriors had effective sample sizes (ESS) > 100. However, the 'prior', 'posterior' and 'coalescent' ESS's remained < 100 even after this long run. We interpret this as an effect of the complexities of fitting a single demographic history to the coalescent pattern of several independent loci.

To discard the possibility that family-induced genetic structure could produce a false signal of population size reduction (Chikhi *et al.* 2010; Heller *et al.* 2013; Städler *et al.* 2009), we reran demographic tests #1 and #2 using the 'full dataset' (*C. polykomos*: 52 individuals, *P. b. temminckii*: 72 individuals) and the reduced 'non-related dataset', comprising non-related individuals only (*C. polykomos*: 22 individuals, *P. b. temminckii*: 23 individuals). We have not done the same for the EBSP analysis because it is a Bayesian approach like demographic tests #2 and therefore, subject to similar effects of the genetic structure in its results.

3. Results

3.1 Fecal Samples for Genetic Analyses

A total of eight *C. polykomos* and six *P. b. temminckii* social groups were sampled (Fig. 1). By sampling one social group of each taxon per forest fragment (Fig. 1), we collected 380 fecal samples. After removing the repeated individuals and the low quality DNA samples from the dataset we analysed 52 *C. polykomos* individuals (11-14 loci, 97.2% complete genotypes, mean quality index (Miquel *et al.* 2006) of 0.84) and 72 *P. b. temminckii* individuals (10-13 loci, 96,5% complete genotypes, mean quality index of 0.77). When we removed the significantly related individuals from the dataset none of the loci showed evidence for null alleles, genotypic linkage disequilibrium or deviations from Hardy-Weinberg equilibrium.

3.2 Genetic Structure at the Population Level

Analysis of population structure using STRUCTURE and BAPS revealed concordant results. The analysis of *C. polykomos* individuals using STRUCTURE revealed K=4, but all individuals had approximately identical admixture proportions, suggesting an absence of overall population structure (Fig. 2a). BAPS also strongly supported the absence of population genetic differentiation (K=1, p=0.99; Fig. 2b). For *P. b. temminckii,* although ΔK was highest for two clusters, the admixture proportions were again approximately identical among all individuals (Fig. 3a), so STRUCTURE suggested an absence of genetic structure (also supported by the highest mean LnP(K) for K=1). BAPS supported the lack of differentiation, as one single genetic entity (K=1) best explained the dataset (p=0.98) (Fig. 3b). These data, taken together, strongly indicated that for both species, all

individuals in Cantanhez National Park are part of the same genetic unit.

3.3 Fine-scale Genetic Structure

Mantel tests provided contrasting results for the two taxa. *C. polykomos* showed an overall and significant negative correlation between pairwise relatedness and geographic distance (Fig. 4), implying that geographic distance plays an important role in shaping the distribution of genetic diversity in this population. In contrast, we found no correlation between dyadic relatedness and geographical distance in *P. b. temminckii* (Fig. 4). This was particularly true for entire social groups and for females only (Fig. 4a and 4b). For *P. b. temminckii* males, there was a tendency for a negative correlation, which may not have reached significance due to the limited number of males in the sample (Fig. 4c)

For the first distance class of the spatial autocorrelation analysis (corresponding to within group dyads), all individuals of both species were significantly positively related to each other (except *P. b. temminckii* males), as is expected from group-living organisms (Fig. 5). In *C. polykomos*, with all individuals included (N=132-133 dyads) and for males only (N=23-24 dyads), relatedness was higher than expected at medium distances (Fig. 5a and 5c, respectively), a pattern that was not observed for females (N=23-24 dyads, Fig. 5b). At distances further than 14.1 km all *C. polykomos* were less related than expected at random (Fig. 5a). Because females constitute most of the *P. b. temminckii* sample, the spatial autocorrelation patterns of females ($N_{females}$=213-214) were similar to the ones obtained for entire social groups ($N_{social\ group}$=319-320). They were still significantly more related than expected at random in the second distance interval, corresponding to individuals belonging to neighbouring groups. We also found that individuals were negatively related at further but not at the maximum distances (Fig. 5a and 5b). The relatedness between pairs of *P. b. temminckii* males (N=13) was not higher or lower than expected at random, for any of the distance classes (Fig. 5c).

3.4 Demographic History

Demographic Test #1

The heterozygosity excess test did not show evidence of a departure from mutation drift equilibrium across mutation models for *C. polykomos* (Table 1). For the *P. b. temminckii,* both the infinite allele model (IAM) and the two phase model (TPM)(78%) showed clear signals of a demographic bottleneck for both datasets ("full" and "non-related"). However, the TPM (95%) was only able to detect an excess of heterozygotes for the "non-related" dataset and the SMM did not detect any heterozygosity excess.

Demographic Test #2

Results from MSVAR 1.3 showed evidence for a population bottleneck in both species, even though the signal was stronger for *P. b. temminckii*. We found bottleneck

evidence using both datasets ("full" and "non-related") in the two colobus species, although the signal was stronger using the "full" dataset (Table A2). Here, we will only refer to the results obtained with the "non-related" dataset in order to discard the potential effect that the kin-based structure might have on increasing the true signal of the demographic bottleneck. The multivariate potential scale reduction factor (~1) and the estimates of the corrected scale reduction factors (CSRF) and their 97.5% quantiles for each parameter (<1.20) indicated acceptable convergence across the four independent runs. For *C. polykomos,* we obtained some overlap between the posterior distributions of N_0 and N_1 (Fig. 6a, solid lines), which was also evident by the 90% highest posterior density intervals (HPD90%; N_0: 3 – 6 166 individuals, N_1: 724 – 87 096 individuals, Table 2). However, the N_0 and N_1 medians were substantially different: the estimated current effective population size was between 251 and 575, whereas the estimated ancestral population size was one order of magnitude larger (5 012 to 5 623). This was further supported by the N_0/N_1 ratio with median values between 0.05 and 0.08 (HPD90%: 0.02 x 10^{-10}-0.70, Table 2). We also found a wide posterior distribution for the time since the demographic change occurred (Fig. 6b). However, even though the posterior distribution was rather wide, it clearly identified recent times as the most likely period during which the bottleneck took place. For instance, even though the prior had a median value around 10 000 years ago the posterior's median values ranged from ca. 1 000 to 3 000 years in the past (Table 2). In fact when the posterior was represented in a natural rather than log scale the mode is close to zero, indicating thus a very recent bottleneck.

We obtained an even stronger signal of genetic bottleneck for *P. b. temminckii*, with posterior distributions for N_0 and N_1 showing very limited overlap (Fig. 7a, solid lines). The median values across runs for the N_0 were 159 to 371 (HPD90%: 3 - 4 074, Table 2). For the N_1 we obtained median values that were larger by two orders of magnitude (13 490 – 15 136) with HPD90% intervals ranging from 3 540 to 60 256, suggesting that this species have undergone a strong population reduction (Table 2). The N_0/N_1 for *P. b. temminckii* confirmed this evidence, as median values ranged from 0.02 to 0.03 (HPD90%: 2.27 x 10^{-6}-0.12, Table 2). As with *C. polykomos*, the demographic tests #2 also suggests a very recent bottleneck, with median values for the estimated age of the demographic collapse that ranged from ca. 1 500 to 3 500 years ago and HPD90% intervals were between 27 and 52 481 (Table 2).

The results of the first BF analysis strongly favoured the H1 scenario for both species (*C. polykomos* BF = 3 309, *P. b. temminckii* BF = 3 198), attributing the population decline to very recent events, most likely related with the recent anthropogenic use of the forest (Fig 8a). The remaining hypotheses were clearly rejected, excluding the possibility that the decrease of the colobus populations in CNP was related with the initial human arrival to this peninsula (*C. polykomos* BF = 1.22, *P. b. temminckii* BF = 1.22), past dry periods during which contraction of the forest occurred (*C. polykomos* BF = 0,29, *P. b. temminckii* BF = 0.37), the last glacial maximum (*C. polykomos* BF = 0.04, *P. b. temminckii* BF = 0.03) or to more ancient events (*C. polykomos* BF = 0.02, *P. b. temminckii* BF = 0.007) (Fig. 8 and 9). This analysis is confirmed by the other BF analysis, where time was not divided into different time periods but rather divided into similar 10 year long periods up to 100 years ago and into 100 year long periods onwards (Fig. 8b and 8c). For both species, the BF values decrease from very high values in the very recent past to low values beyond several thousands of years.

Demographic Test #3

The second Bayesian method (EBSP) that we applied to infer the demographic history of the CNP colobus monkeys, supported the trend of demographic bottleneck for both species and the stronger bottleneck for *P. b. temminckii,* similar to the demographic tests #2 results. The demographic tests #3 differed substantially from the demographic tests #2, however, in the estimated posterior distributions for N_1 and T, indicating weaker and older bottlenecks (Fig. 9). Demographic tests #3 suggested that *C. polykomos* decreased from an ancestral population size of approximately 876 individuals to a current population of 125 (Fig. 9a; mean/median values). The beginning of this demographic collapse is estimated to be older than suggested by demographic tests #2, dating 15 000 years back. For the *P. b. temminckii,* we again obtained a stronger bottleneck signal, with the population decreasing from approximately 1 930 individuals to a current size of 223 individuals (Fig. 9b). According to the demographic tests #3, this population started to collapse at around 16 000 years back. This analysis also suggested an increasingly rapid decline from 1 100 generations ago (equivalent to 5 500 years back in time) (Fig. 9b).

4. Discussion

4.1 Absence of Genetic Structure at the Scale of Cantanhez National Park

Overall, we found no strong signal of population structure in the two colobus monkeys in Cantanhez National Park. Both clustering approaches (spatial and non-spatial) were concordant. The only substantially discordant results that we found were obtained when we compared the 'full' with the 'non-related' datasets, which was particularly evident for *P. b. temminckii.* When the most related individuals were removed, both approaches agreed in identifying a single genetic cluster. The absence of major population genetic differentiation suggests that all individuals of each species are part of the same genetic unit, indicating an absence of major barriers to dispersal, at least up to the recent past. This does not necessarily mean that all individuals come from a single founder population. In fact, analyses of the mitochondrial DNA have suggested that the population of *P. b. temminckii* from Cantanhez results from the secondary contact between divergent lineages (Minhós et al 2013b). What our population genetic structure results indicate instead is that, even if the populations were differentiated in the past, enough time has passed without major dispersal barriers in Cantanhez and consequently the nuclear genome had become homogenized.

4.2 Spatial Genetic Structure in a Fragmented Habitat

While we could not identify discrete units within the Cantanhez National Park we found that genetic diversity followed an isolation-by-distance pattern in *C. polykomos*, whereas this was not the case for *P. b. temminckii*. The negative correlation between

pairwise relatedness and geographic distance found in *C. polykomos* males and females suggests that *C. polykomos* individuals tend to disperse in a stepwise manner (i.e. in neighbouring groups) and that gene flow is possible (or has been in the recent past) throughout the park with no major barriers. Despite the absence of continuous forests in the park, some connectivity may still be maintained between the patches or individuals may move on the ground across the matrix to reach new forested areas. The ability of *C. polykomos* to persist in fragmented forests has already been noted in previous studies (Dunbar 1987; Struhsaker 2005; Onderdonk & Chapman 2000). Additionally, the fact that a linear correlation between relatedness and geographic structure exists for both sexes agrees with previous evidence for dispersal mediated by both sexes in this population (Minhós *et al.* 2013b). The spatial autocorrelation analysis showed some degree of fine-scale genetic structure. *C. polykomos* males were more related than expected at random up to distances of 9-12km, contrary to the females that were only more related that expected at random at the intra-group level (first distance interval). Interpretation of such results should be done with caution but this may reinforce the evidence that, in general, males are able to disperse longer distances than females. At further distances (14 to 30km) the analysis with all individuals included showed that individuals are negatively related, as expected when dispersal is conducted in a stepwise manner. At this stage, we should add that the patterns described above might either reflect ongoing or former connectivity among specific forest patches. Direct observations of dispersal and mating events are therefore needed.

In *P. b. temminckii*, the lack of an isolation-by-distance pattern (IBD) suggests that there might be other factors besides geographical distance shaping population genetic structure or that migration events happen at larger distances than in *C. polykomos* and consequently do not generate an IBD pattern within the Cantanhez park. Indeed, high gene flow or an absence of gene flow can both lead to a lack or limited correlation between genetic and geographic distance (Hutchison & Templeton 1999). For *P. b. temminckii* males, our interpretation must be even more cautious due to their limited number and distribution in our sample. However, *P. b. temminckii* females seem to have some dispersal restrictions contrary to *C. polykomos* females. Red colobus females were more related than expected at the first distance interval involving individuals from adjacent groups (approx. 1.5km). This corresponds specifically to the pairwise relatedness between dyads belonging to the Focal and Madina social groups (see Fig 1). The females from these two groups may be the reason for the disruption of the IBD pattern in this female dispersing population (Minhós *et al.* 2013b), illustrating how a single outlying pattern of dyad relatedness between specific groups can bias the overall trends. This evidence is supported by the higher number of related females between the Focal and Madina social groups, compared to the other social groups (Table 3). Several episodes of humans hunting of *P. b. temminckii* individuals have been observed in Madina forest (T. Minhós, personal observation; K. Hockings personal communication). In contrast, the Focal group inhabits an area that overlaps with a tourist lodge (T.Minhós, personal observation), which may confer some level of protection against human hunting. Hunting was never observed nor reported in this social group, contrary to all other social groups sampled. It is possible that *P. b. temminckii* females from Madina preferably disperse into the geographical close and safer Focal group, hence adopting behavioral strategies to cope with the anthropogenic disturbance of the forest. In fact, recent socio-genetic analyses on the Focal group have demonstrated unexpected

cooperation among unrelated females that might result from an increased population density and intragroup competition in this particular social unit (Minhós et al. 2015). Moreover, similar dispersal dynamics among red colobus females living in highly fragmented habitats have also been described by Starin (1994) in Abuko Nature Reserve in The Gambia. Nevertheless, the disruption of the IBD pattern in this female dispersing system could be mainly a consequence of a more restricted dispersal by females, that are usually more vulnerable while dispersing and tend to disperse shorter distances than males (Waser 1985). The absence of an IBD pattern, together with the spatial autocorrelation analysis indicates that, in this population, gene flow is not correlated in a simple way with geographical distance. The fact that this fine-scale structure was not found for *C. polykomos* females (where geographic distance plays a major role), suggests that *P. b. temminckii* females could be more susceptible to the forest disturbance.

4.3 Past Demographic Changes

Since the 1990s and the seminal studies of Slatkin and Hudson (1991) and Rogers and Harpending (1992) on mismatch distributions, population geneticists have been increasingly interested in reconstructing the history of populations from molecular data. During the following twenty years methods have greatly improved. The demographic analyses that we performed showed both a series of consistent results but also some results that were at least apparently not in full agreement. Some of the differences are probably due to the fact that we used different approaches, which all have their own limitations and do not capture the same signals from the data. The demographic test #1, implemented in Bottleneck, is based on determining whether a population is at mutation-drift equilibrium under several mutation models. It is limited by the fact that it uses only a small amount of the genetic information but is well established and allowed us to test for a departure in the direction of a bottleneck or an expansion. The demographic test #2, implemented in MSVAR, is much more efficient at retrieving information from the genetic data but assumes a very specific demographic and mutation model (Beaumont 1999). If the demographic history of the population of interest is very different from that assumed (one single demographic event) it may provide misleading results. Finally the demographic test #3, the EBSP approach, allows the reconstruction of more complex demographic histories. However, this method has mostly been used on sequence data and has not been yet applied to many microsatellite data sets and little simulation work has been done to validate it for these types of genetic markers. We observed slow convergence using the demographic test #3, and we conclude that it is hard to fit an unconstrained demographic model to multi-locus microsatellite data. We therefore consider these three approaches as complementary.

Altogether, we found a consistent signal of population collapse for both species using the two Bayesian methods (demographic tests #2 and #3). As expected, the excess of heterozygosity tests (demographic test #1) were only able to detect a signal of population decrease in some of the data sets. More specifically a bottleneck was detected for *P. b. temminckii* and not for *C. polykomos*. This is not necessarily surprising given that these tests only compute the expected heterozygosity based on the observed number of alleles conditional on the sample size. In fact, similar results have been described in many studies (Craul *et al*. 2009; Olivieri *et al*. 2008) and have also been observed with simulated data. Demographic test #1 can fail to detect a population size

change, even in populations that are known to have decreased. Given the results obtained with both demographic tests #2 and #3, this negative result is probably a consequence of the low statistical power of this approach (Girod et al. 2011; Peery *et al.* 2012).

The fact that demographic tests #2 and #3 (MSVAR and EBSP) suggested a demographic collapse for both species and were concordant in detecting a stronger bottleneck for *P. b. temminckii* is very persuasive. It is also reassuring to see the demographic test #3 identify a demographic history similar to that assumed by the demographic test #2 (i.e. an ancient constant population that suffered a bottleneck). Also, both approaches estimated similarly low values of N_0 (the current population size) for both species but appeared to differ in the estimation of N_1 (the ancestral population size) for *P. b. temminckii*. The demographic test #3 estimated N_1 values that were substantially smaller than those estimated with demographic test #2, even though the posterior distributions of the methods do overlap. Additionally, both methods were concordant in suggesting that *P. b. temminckii* started to decrease slightly earlier than *C. polykomos*, even though these estimates overlap extensively. Comparing the dating of the demographic collapse between the two methods is not straightforward. The demographic test #3 estimated rather ancient times for the demographic change, whereas demographic test #2 favours a very recent bottleneck (see BF analyses). It is difficult to identify the exact reasons why the two methods should differ here, but it may warrant more study. It is, however, important to emphasize that the two approaches differ in several ways. The demographic test #2 assumes a very specific model of population size change and then estimates the parameters of that specific model that best explain the data. The demographic test #3 is different in that the demographic history is much less constrained. This has the advantage of allowing for multiple population size changes or non-monotonous changes. This greater flexibility may come at the cost of precision when the true demographic history is in fact simple, although this requires further study. We suggest that some simulation work might be useful. In particular, the demographic test #3 has been little used and validated with microsatellite data (Allen *et al.* 2012; Andersen *et al.* 2013; Molfetti *et al.* 2013), even if it has been widely used and tested with sequence data (e.g. Barnes *et al.* 2007; Heller *et al.* 2008; Stiller *et al.* 2010). On the contrary, the capacity of demographic test #2 to detect demographic bottlenecks and expansions has already been tested and validated (Girod *et al.* 2011) and extensively applied to natural populations of several species (e.g. red colobus: Allen *et al.* 2012, orang-utans: Sharma *et al*. 2012; mouse lemurs: Olivieri *et al*. 2008; sportive lemurs: Craul et al. 2009; sifakas: Lawler 2011; Quéméré *et al.* 2012; polecats: Costa *et al.* 2013; sea turtles: Plot *et al*. 2013). At this stage it is difficult to properly assess the discrepancy (although the demographic test #2 posteriors are rather wide and encompasses much of the demographic test #3 posteriors) and how much of it is caused by the methodological differences of the two approaches.

Finally, we must note that all the methods used here assume that the populations sampled are isolated (i.e. the non structured Wright-Fisher model). Several recent studies have shown that genetic structure can generate spurious population size change (Chikhi *et al*. 2010; Heller *et al.* 2013; Städler *et al.* 2009; Wakeley 1999). Here we tried to account for that following the suggestions of Chikhi *et al*. (2010) and Heller *et al.* (2013) who showed that this bias could be minimized by testing several sampling schemes (i.e. by conducting the analyses using different number of individuals from each

sampled location). The bottleneck signals identified here seemed robust to the sampling scheme, and we are therefore confident that the bottleneck signals are unlikely due to structure alone. Biologically they are the likely signature of a significant demographic decrease and of a reduction of gene flow (Broquet *et al.* 2010).

4.4 Conservation Implications

Methodological discrepancies aside, the results come in agreement with our expectations, given the species socio-ecological features. As arboreal species, both have been impacted by the recent forest exploitation, as both showed a recent severe demographic collapse and exist in very low numbers in the park. However, *P. b. temminckii* shows a lower ability to cope with such habitat disturbance: it appears to have suffered a stronger genetic bottleneck than *C. polykomos.* Also, it appears that females have some restriction on dispersal and finally we could not find any social groups in more human-disturbed forest patches (north of the park, Fig.1).

As observed during previous visits, *P. b. temminckii* groups are decreasing or even disappearing from areas where they used to be found. Whereas *C. polykomos* existed in almost all patches, *P. b. temminckii* were not found north of Iemberem (centre of the park, see Fig 1), where the forest is more fragmented and illegal hunting and logging is less controlled. While local communities have historically preserved forest fragments and their biodiversity south of Amidara, in the northernmost forests, land use and resource exploitation were not controlled (Temudo 1998; Temudo 2009). Some *P. b. temminckii* social groups were detected around Amidara in 2008 and 2009 but not in 2010 (T. Minhós, personal observation). The lack of samples in the north of the park may suggest either lower density or even the absence of this species in these more altered areas. Even if *P. b. temminckii* groups are still present in the deeper areas of these forests, the fact that they were not found together with *C. polykomos* in more degraded habitats supports the evidence of their higher susceptibility to this threat.

The demographic results are alarming from a conservation standpoint and agree with past direct observations in the park: colobus populations are small and have been decreasing. We estimated current effective population sizes below 500 individuals for both species. While these numbers should not be taken at face value, they are low and given the increasing isolation of these populations from the rest of the species distribution, they may actually be overestimates. Indeed the population may maintain part of the genetic diversity of the wider population when it was connected to other regions. Authors have suggested that an effective size below 500 might not be sufficient to maintain the evolutionary potential of a population (Frankham *et al.* 2014). We do not think that one number such as 500 is likely to apply to all vertebrate species and therefore we are cautious with our interpretations. However, field observations also suggest that the two colobus species are likely to suffer population declines in the future, as social groups of both species have been observed disappearing and we could not find red colobus north of the park.

The most likely time since the demographic change occurred, indicated by BF, also support the urgency for conservation initiatives directed at these populations. Even though the estimated MSVAR medians fell between 1 000 and 3 500 years in the past, the Bayes Factor tests supported very recent onset of decline (< 2-500 years ago). At this stage, the genetic data suggest that the two species suffered from a decline that is likely

related to recent anthropogenic use of the forest resources (forest loss/fragmentation and hunting). For example, in Tanzania, the recent anthropogenic forest disturbance have also been appointed as the main cause maintaining the genetic differentiation among Udzungwa red colobus populations (*P. gordonorum*), stressing the high susceptibility of red colobus to human-induced changes in the habitat (Ruiz-Lopez et al 2015). Both colobus populations are currently of similar and very low size. However, in the past, the population of *P. b. temminckii* was larger than that of *C. polykomos*, possibly as a consequence of living in bigger social groups. The larger social groups of *P. b. temminckii* probably demanded largest areas of undisturbed forest. Additionally, their bigger group sizes also make this species more conspicuous to human hunting (Ferreira da Silva 2012). Moreover, the fact that both *C. polykomos* males and females are able to disperse and to live in smaller groups most likely favours their ability to disperse longer distances and establish social groups in smaller and more disturbed forest patches (Minhós et al. 2013b; Onderdonk & Chapman 2000). On the other hand, the fact that dispersal in *P. b. temminckii* is restricted to females (Minhós et al. 2013b) may already constrain the dispersal of this species to shorter distances, as it is widely recognized that in general males disperse greater distances than females (Waser 1985). This dispersal system, together with the fact that *P. b. temminckii* individuals are unable to maintain small social groups, most likely hinders their abilities to cope with high levels of habitat disturbance. Altogether, these features of *P. b. temminckii,* combined with its inability to socially and ecologically adapt to changing environments most likely contributed for their stronger demographic collapse in CNP.

Our results suggest that the long-term survival of both species in CNP is already compromised if conservation actions are not implemented immediately. Cantanhez is the last area in Guinea Bissau where colobus exist in larger but decreasing numbers and with some remaining suitable habitat (IBAP 2007). While it is imperative that the existing corridors are maintained in order to ensure gene flow through the park, it is even more important that suitable forest habitat is restored, especially in the northern part of the park, in order for *P. b. temminckii* groups to re-establish. In addition to forest loss and fragmentation, both colobus are also targets for hunting and bushmeat consumption (Costa *et al.* 2013a; Minhós *et al*. 2013a). Although this threat seems to affect *P. b. temminckii* more than *C. polykomos* (Minhós *et al*. 2013a), it will certainly accelerate the population decrease of both species. Therefore, future conservation plans should not only consider habitat connectivity and rehabilitation. Law enforcement towards illegal hunting may also be necessary but should not be done by targeting the poorest community in the park but rather the wider community creating a demand for bushmeat. This may require international actions in the main cities where richer populations may create an unsustainable market for an endangered primate (Brashares *et al.* 2011).

The conservation situation of both species is critical in Guinea Bissau and we know close to nothing about the situation of other populations in West Africa. While some East African colobus populations have been genetically monitored (Allen *et al*. 2012; Harris *et al.* 2009; Miyamoto *et al.* 2013; Ruiz-Lopez et al. 2015), no similar study has been conducted for either of the remaining West African colobus populations. Most of them exist in countries with long-lasting political instability and sporadic surveys indicate a critical scenario for West African colobus monkeys (Oates et al. 2000; Oates 2008a; Oates 2008b; Struhsaker 2005). Our results stress the need to extend conservation

749 genetic and socio-ecological studies to other West African colobus populations before
750 populations of these species decrease to unsustainable numbers.
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Data Accessibility

Microsatellite data deposited into Dryad.

Authors Contributions

T.M., M.W.B., C.S., L.V. and C.C. Research concept and design; T.M., M.F.S. and R.S. Collection and/or assembly of data; T.M., L.C., M.F.S., R.H., M.W.B., Data analysis and interpretation; T.M. and R.H. Statistical analysis; T.M. Writing the article; T.M, L.C., C.S, M.F.S., R.H., C.C. M.W.B.; Critical revision of the article and T.M., L.C., C.S, L.V., M.F.S., R.S., R.H., C.C. and M.W.B. Final approval of the article

Tables

Table 1. Results (p-values) of the heterozygozity excess test performed for the infinite allele model (IAM), two-phase model (TPM) and stepwise mutation model (SMM) in BOTTLENECK.

Mutation Model	*C. polykomos*		*P.b. temminckii*	
	Full Data	Non-Related	Full Data	Non-Related
IAM	0.15308	0.13379	**0.00006**	**0.00006**
TPM (78%)	0.31287	0.21313	**0.00085**	**0.00061**
TPM (95%)	0.86621	0.31287	0.18787	**0.03406**
SMM	0.97906	0.61957	0.60657	0.08386

Significant results are in bold.

Table 2. Posterior distributions of the ancestral population size (N_0), current population size (N_1), their ratio (N_0/N_1) and the time since the demographic change (T), for the "non-related" databases, using MSVAR 1.3

	C. polykomos	Run 1	Run 2	Run 3	Run 4	Overall
N_0	Mean	490	398	282	178	316
	Median	575	501	371	251	417
	HPD90%	9 – 6 166	21 – 4 467	7 – 4 365	3 – 3 981	7 – 4 898
N_1	Mean	6 918	5 623	6 166	5 754	6 166
	Median	5 623	5 248	5 248	5 012	5 248
	HPD90%	724 – 87 096	933 – 35 481	832 – 52 481	871 – 33 884	831 – 46 773
N_0/N_1	Mean	50 758	8	74	381	-
	Median	0.081	0.082	0.05	0.06	-
	HPD90%	$3.73x10^{-10}$-	$1.44x10^{-6}$-	$2.52x10^{-10}$-	$1.02x10^{-10}$-	-

		0.70	0.54	0.35	0.44	
T	Mean	4 266	3 715	2 630	1 585	2 884
	Median	3 090	2 818	1 995	1 288	2 188
	HPD90%	17 – 398 1072	35 – 776 247	12 – 1 000 000	6 – 380 189	12 – 1 148 154
	P. b. temiinckii					
N_0	Mean	371	347	204	159	257
	Median	468	398	295	229	339
	HPD90%	21 – 4 074	28 – 3 311	3 – 3 715	3 – 3 162	8 – 3 890
N_1	Mean	15 488	13 804	13 804	13 490	14 125
	Median	15 136	13 804	13 804	13 490	14 125
	HPD90%	3 802 – 60 256	3 548 – 53 703	3 631 – 54 954	3 388 – 52 481	3 631 – 56 234
N_0/N_1	Mean	0.043	0.034	0.042	0.030	-
	Median	0.031	0.022	0.029	0.016	-
	HPD90%	$5.31x10^{-5}$-0.12	$5.35x10^{-6}$-0.11	$1.80x10^{-4}$-0.12	$2.27x10^{-6}$-0.10	-
T	Mean	3 020	2 630	1 622	1 318	2 042
	Median	3 467	2 818	2 042	1 622	2 455
	HPD90%	141 – 52 481	166 – 38 019	27 – 46 774	29 – 36 308	63 - 48 978

Table 3. Number of inter-group female dyads related above the half-sibling level (half-siblings, full-siblings and parent-offspring) for the Focal and Madina social groups. The numbers in bold correspond to the intra-group dyads.

Social Groups	Focal	Madina
Focal	**53**	20
Madina	20	**27**
Cambeque	10	13
Cangode	16	11
Cungha	20	7
Muna	2	0

Figures

Fig. 1. Map of the colobus groups sampling locations in Cantanhez National Park (provided by GPC and INEP). Green – forest; light green – mangrove; dark green – palm trees; light orange – woodland savannah; orange – grassland savannah; blue – rice fields; light yellow – crops; brown – tannes; black lines – main dirt roads. Black circles: *C. polykomos* sampled groups; red circles: *P. b. temminckii* sampled groups. 1 – Cancira; 2 – Amidara; 3 – Deep Amidara; 4 – Focal and Neighbor; 5 - Madina; 6 – Cambeque; 7 - Cangode; 8 – Muna; 9 – Cungha.

Fig. 2. Bayesian population genetic clustering of *C. polykomos* using SRUCTURE (non-spatial; a) and BAPS (spatial; b). Different colours represent different genetic clusters and the names represent different social groups. With STRUCTURE, each column represents a different individual. With BAPS, each polygon represents one social group and each cell of the tessellation corresponds to the physical neighbourhood of an individual.

Fig. 3. Bayesian population genetic clustering of *P. b. temminckii* using SRUCTURE (non-spatial; a) and BAPS (spatial; b). Different colours represent different genetic clusters and the names represent different social groups. With STRUCTURE, each column represents a different individual. With BAPS, each polygon represents one social group and each cell of the tessellation corresponds to the physical neighbourhood of an individual.

Fig. 4. Correlation (r) between pairwise relatedness and geographic distance (Km) for *C. polykomos* and *P. b. temminckii*. Each dot represents a dyad of individuals. Mantel tests were performed for males and females together (a), only females (b) and only males (c).

Fig. 5. Spatial autocorrelation using pairwise relatedness and pairwise geographic distance (Km) for *C. polykomos* and *P. b. temminckii*. Stars (*) represent significant deviation from the 95% interval (dashed grey lines) to the null hypothesis of relatedness=0. Analyses were performed for males and females together (a), only females (b) and only males (c).

Fig. 6. Demographic bottleneck detected in *C. polykomos*, using MSVAR 1.3 Black solid lines: posterior distributions of the current effective population size (N_0) for four independent runs; grey solid lines: posterior distribution of the ancestral population size (N_1) for four independent runs; green solid lines: posterior distribution for the time (in years) at which the demographic change have occurred (T), for four independent runs. Dashed lines represent prior distributions used for the three estimated parameters. All values are represented in a $\log_{10}$ scale.

Fig. 7. Demographic bottleneck detected in *P. b. temminckii*, using MSVAR 1.3 Red solid lines: posterior distributions of the current effective population size (N_0) for four independent runs; orange solid lines: posterior distribution of the ancestral population size (N_1) for four independent runs; green solid lines: posterior distribution for the time (in years) at which the demographic change have occurred (T), for four independent runs. Dashed lines represent prior distributions used for the three estimated parameters. All values are represented in a $\log_{10}$ scale.

Fig. 8. Most likely period for the start of the population decrease. Solid lines (black*: C. polykomos*, red: *P. b. temminckii*) indicate the values of the BF for fixed intervals of 10 between 0 and 100 years and of 100 between 200 and 100 000 years for both study species. The BF measures the likelihood of the hypothesis that the populations started to

decrease at a given time interval (H1) versus all other time periods (H2). BF = 1 indicates that H1 and H2 are equally probable, 1< BF > 3 give only slight support for H1, 3 < BF > 10 indicate moderate support for H1 and BF > 10 strongly support H1 (dashed horizontal lines) (a, b). We tested for the following five scenarios (vertical dashed lines): H1 (0 - 200 yrs) – recent anthropogenic forest exploitation; H2 (200 – 1 000) – human colonization of CNP; H3 (1 000 – 6 000) – sequence of dry periods in West Africa; H4 (19 000 – 27 000) – last glacial maximum; H5 (27 000 – 100 000) – ancient events (c).

Fig. 9. Demographic history using Extended Bayesian Skyline Plots (EBSPs). Inferred means (thick grey line), medians (thick black lines) and 95% HPD interval (thin grey lines) of the population size parameter (N_e x generation time) over time (measured in generations). Panel (a) shows *C. polykomos*; panel (b) shows *P. b. temminckii*.

Fig. A1. Bayesian population genetic clustering of *P. b. temminckii* full dataset using SRUCTURE (non-spatial; a) and BAPS (spatial; b). Different colours represent different genetic clusters and the names represent different social groups. With STRUCTURE, each column represents a different individual. With BAPS, each polygon represents one social group and each cell of the tessellation corresponds to the physical neighbourhood of an individual.